Cornell iGEM Phen Green SK Metal Sequestration Experiments:
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4 strains x 1 metal for 3 strains, 3 metals for control x 3 replicates = 18 total cultures.

4 strains:
AI+R (Metallothionein+CBP4)
AI+AG (Metallothionein + merT/merP)
AI+AF (Metallothionein + nixA)
pC13AS (BL21 expressing crtI, used as a BL21 control)

Day 1: Preparation
1. Start 10 mL overnight cultures of each of the above strains in LB+ appropriate antibiotic (Cm, Amp, CM+Amp) + 1x Arabinose
2. Make appropriate metal solutions to use on day 2.

Day 2: Sampling
1. Measure OD of each culture using UV spec and record.
2. Add 10 mL of LB + Arab + Antibiotic + 0.2 mM appropriate metal to each culture:

pC13AS in Pb
pC13AS in Hg
pC13AS in Ni
AI+R in Pb
AI+AG in Hg
AI+AF in Ni

3. Take out ~100 uL of culture and put in a labeled tube and in the freezer.
4. Sample each culture every hour for 12-16 hours by taking out 100uL and freezing.

Day 3: Measurement

1. Prepare 100x stock of Phen Green SK dipotassium salt by dissolving 0.139 mL of reagent in 1 mL DMSO. This gives a final 100x concentration of 0.2 mM and a measurement concentration of 2 μM. **Note: Phen Green SK is light sensitive and unstable. Should be used within 30 days and stored in the dark at -20C.**
2. Spin down samples at 12000xg
3. Combine 75 μL sample supernatant, 73.5 μL PBS, and 1.5 μL 100x Phen Green into the wells of a 96 well plate.
4. Measure fluorescence of samples with excitation wavelength of 507 nM and emission wavelength of 532 nM.
5. Compare fluorescence values with a standard curve.
Note: Fluorescence of phen green will decrease with increased metal concentrations. This dye is non-specific, so high concentrations of other metals will confound results.